Study on the Treatment of Toluene Waste Gas by Biotrickling Filter System based on High-throughput Sequencing

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Abstract: In this study, the self-designed biotrickling filter (BTF) was used to treat toluene waste gas, and the activated sludge was used as the bacteria source to directly biofilm culturing. The performance of the biofilm culturing stage of BTF and the effect of BTF on the removal efficiency of toluene in the stable operation stage were explored. Furthermore, High-throughput sequencing was used to analyze the total bacterial diversity of the bacterial community attached to the biofilm. The experiment shows that the system temperature is 22-28 ℃, the toluene intake is 15 L/min, the toluene import concentration is 100~500 mg/m3, the nutrient spray rate is 60 ml/min, activated sludge can finish microbial acclimation in 30 days, and the removal efficiency is maintained at about 90%. The removal efficiency is still stable at around 90% by changing the toluene intake by 5~25 L/min, the toluene import concentration of 100~800 mg/m3 and the nutrient spray rate of 10~100 mL/min. The toluene import concentration increased to 800 mg/m3, and the removal efficiency decreased. But the removal rate could quickly reach 96% or more after the concentration was lowered. High-throughput analysis of microbial colonies showed that Proteobacteria are the main dominant species, and Gammaproteobacteria is the most abundant of the proteobacteria. In addition, the Simpson index was 0.983319, the Shannon index was 7.72, and the Chao1 and ACE indices were both 1205.00, indicating that the microbial community diversity was high and the community richness was also a higher level.

1. Introduction

Rapid urbanization and industrialization contribute to the growing emissions of air pollutants into the environment. Volatile organic compounds (VOCs), that are classified as major contributors to air pollution. They contribute both indirectly as ozone/smog precursors and directly substances toxic to the environment[2,11,23] . According to the health agencies, VOCs are considered as human carcinogens and longtime exposure to VOCs leads to diseases like reduced pulmonary function, asthma[22]. Also, nasopharyngeal cancer can be caused by long exposure to formaldehyde which also comes into the category of carbonyl compounds. Besides, discharge of VOCs in the environment leads to urban smog and ozone, ozone layer depletion in stratosphere, and the greenhouse effect[12]. VOCs are emitted in large quantities from industries producing paper, paint, solvents, and wood products, as well as from fuel/petroleum industries and pharmaceutical operations, threaten the environment and human health [20,18,16].

The emission of VOCs can be controlled using methods based on physics, chemistry ,and biology. The techniques based on physics include absorption, adsorption, membrane separation, and condensation. High concentrations of VOCs, especially water-soluble compounds can be removed from flue gas streams by absorption using a suitable solvent. The disposal of VOCs and the spent solvent from an absorber are the common problems faced by the absorption processes. Chemical methods for processing VOCs include thermal combustion, catalytic combustion, non-thermal plasma and catalytic oxidation. Thermal oxidation or thermal incineration is suitable for removing VOCs from flue gas streams with a high flow rate and a high concentration of VOCs. More than 99% of the VOCs can be burned by thermal oxidation, typically at high temperatures (>1000℃), which requires additional fuel and temperature resistant materials. Incomplete thermal combustion produces undesirable byproducts such as dioxins and carbon monoxide in the incinerator flue gas. Moreover, noxious byproducts are formed as a result of thermal incineration[14]. Biological technologies such as biofilters (BF), bio scrubbers, and biotrickling filters (BTFs). Biotechnologies are regarded as a cost-efficient and green process[3,9-10]. They are currently recognized as the best available methods for the treatment of low and moderate concentrations of waste gases containing odorous pollutants and VOCs because they are cost-effective and environment-friendly when compared with other physical and chemical technologies[19].

Therefore, this study designed the biological drip filter system to explore the removal efficiency of toluene in the bio-trickling system. The high-throughput sequencing method was used to analyze the total bacterial diversity of the bacterial communities attached to the biofilm, and screen the dominant microorganisms. It provides some theoretical support for the following research.

2. Materials and Methods

2.1. Design of BTF

The main body of the biological drip filter tower system is a cylindrical tower made of plexiglass, with an inner diameter of 180 mm, an outer diameter of 190 mm, a wall thickness of 5 mm, and a total tower height of 926 mm. The sectional area and effective volume of the tower are 2.54×10-2 m2 and 1.27×10-2 m3 respectively. The BTF is divided into four parts, the upper layer is the spray and gas defogging layer; the middle layer and the lower layer are the packing layers with a height of 200 mm and 300 mm respectively. The opening ratio of the separator between each layer exceeds 60%, which acts to uniformly distribute the gas and the spray liquid. Each layer is provided with a gas sampling port and a filler sampling port, and the bottom layer is a circulating nutrient solution

buffer and an outlet. One of the important parameters of the BTF is the performance of the filler. In this experiment, 1cm × 1cm × 1cm cubic polyurethane filler is used(Table 1).

Table 1: Performance parameters of polyurethane filler.

Material	density	poriness /(%)	organic loading /(kg.m-3.d)	Specific surface area /(m2.m-3)	Finished product weight /(kg.m-3)
polyurethane	≈1	97.3	1.1	3.2×104	21

2.2. Nutrient Solution Components of BTF

The composition and concentration of nutrient solution[1,5,7]are essential for the growth of microorganisms(Table 2). The nutrient solution provides essential nutrients for microorganisms and ensures environmental humidity during microbial growth. At the beginning of biofilm culturing, an appropriate amount of glucose was added as a carbon source. As the microbial adaptation time increased, the glucose content was gradually reduced, and finally, the toluene was the sole carbon source, and the removal efficiency of toluene was improved.

Table 2: Composition and content of nutrient solution.

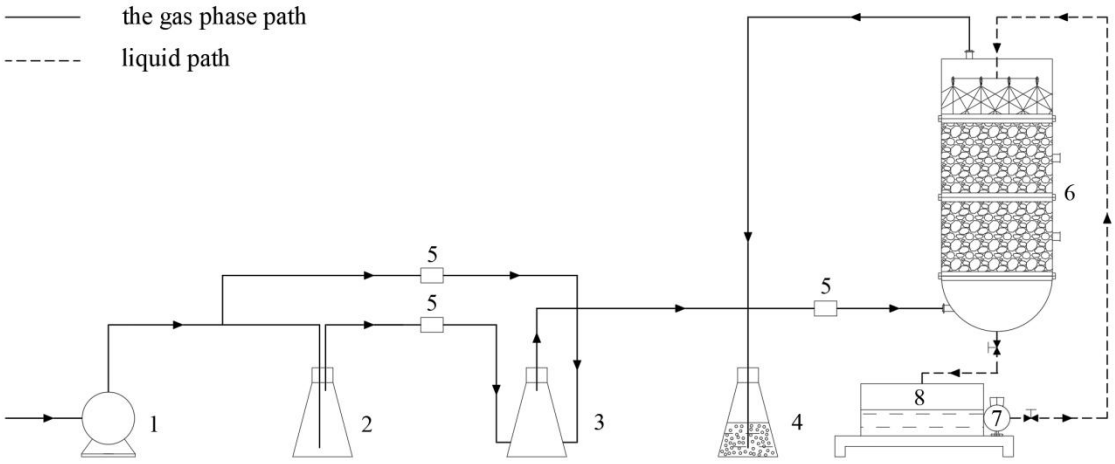
No.	Materials	Chemical formula	content/(g*L^{-1})
1	Glucose-hydrate	$C_6H_{12}O_6$	0.1~0
2	Ammonium chloride	NH_4Cl	0.3
3	Magnesium chloride	$MgCl_2$	0.1
4	Anhydrous calcium chloride	$CaCl_2$	0.1
5	Dipotassium phosphate	K_2HPO_4	0.5
6	Sodium dihydrogen phosphate	NaH_2PO_4	0.5
7	Manganese sulfate	$MnSO_4$	0.3
8	Anhydrous copper sulfate	$CuSO_4$	0.01
9	Zinc sulfate heptahydrate	$ZnSO_4.7H_2O$	0.01
10	Ferrous sulfate heptahydrate	$FeSO_4.7H_2O$	0.01

2.3. Experiment Process

The BTF system consists of two channels of the gas phase path and liquid path, which are composed of the pump and toluene sparger, mixer chamber, spray circulation device and and tail gas buffer device (Figure 1).

Through into the pump, toluene sparger, mixer chamber, and flow meter to simulate different concentrations and different air intake toluene waste gas. The simulated toluene exhaust gas enters the tower body from the bottom of the BTF and passes through two layers of filler loaded with microorganisms and sprayed through the circulating nutrient solution. After that, it is discharged from the top of the biological trickling filter into the exhaust gas buffer device. The circulating

nutrient solution flows out from the bottom and is transferred to the top of the tower body for circulation spraying. During the experiment, the nutrient solution is periodically replaced.



1. into the pump 2. toluene sparger 3. mixer chamber 4. off-gas collector 5. flow meter 6. BTF 7. water pump 8.recirculating nutrient solution

Figure 1: Process flow chart of biological filtration system.

2.4. Data Processing and Experimental Methods

In this experiment, Origin and Excel software were mainly used for data analysis and mapping. QIIME, PICRUSt, GraPhlAn, Krona, and R software were used to process and graph high-throughput sequencing data. The mass concentration of toluene was determined by Shimadzu GC-2014 gas chromatograph(Gasification chamber temperature 150 ℃, chromatographic column temperature 80 ℃, FID temperature 250 ℃, carrier gas N2 flow rate 30.0 mL/min, airflow rate 400 mL/min, H2 flow rate 40.0 mL/min). High-throughput sequencing analysis of microbial samples using the Illumina, Hiseq 2500 high-throughput sequencer. At the same time, bacterial community morphology was observed using a HITACHI S-4800 scanning electron microscope.

3. Results and Discussion

3.1. Study on Biofilm Culturing of BTF

During the biofilm culturing of BTF, the content of glucose in the nutrient solution was gradually reduced according to 0.1~0 g/L, so that the microorganisms gradually adapted to the nutrient environment filled with toluene, and finally toluene as the only carbon source. Therefore, it is necessary to control the toluene import concentration to be low, and gradually increase slightly in the later stage, and the toluene intake and the nutrient solution spray rate are kept within a relatively constant range. The experiment adopted 15 L/min toluene intake and 60 mL/min spray rate. In addition, the control temperature was 22~28 ℃ and the intake concentration of toluene was 100~500 mg/m3. Figure 2 shows an overview of the process of biofilm culturing. The removal efficiency of toluene in the first 3 days was less than 20%, and this stage was the stage of microbial adaptation to toluene. Do not add glucose to the nutrient solution after 3 days. During the period of 3-25 days, the removal rate of toluene increased with the increase of biofilm culturing time. After 25 days, the removal efficiency of toluene was above 90%, and the toluene intake concentration was changed to 100~500 mg/m3, and the removal rate was still maintained at 90%~95%.

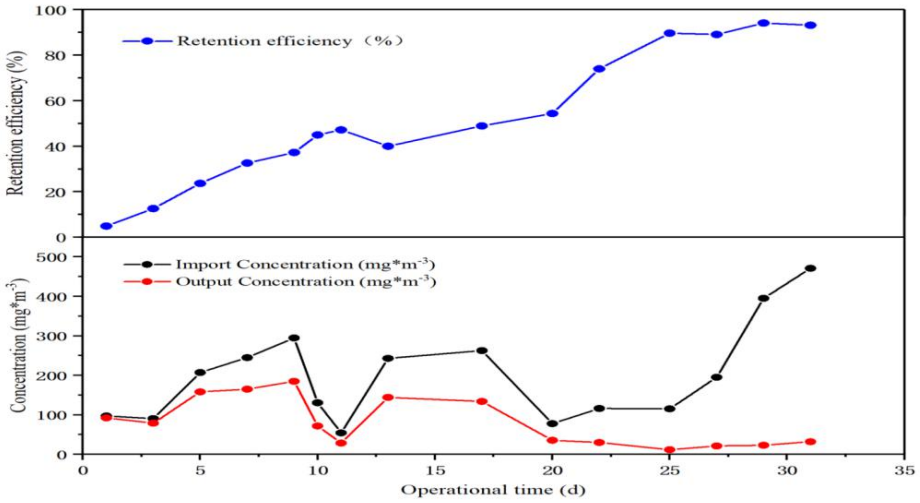


Figure 2: Change of degradation rate of toluene during biofilm culturing.

The results of this phase show that the toluene removal efficiency of the bio-trickling system can reach more than 90% after the 25th day under the condition of 15 L/min of toluene intake and 100-500 mg/m3 of intake concentration. This is consistent with the results of Cheng[7] and Chen[6] studies. However, the Empty Bed Residence Time(EBRT) of their experiment is 96 s and 120 s respectively, while the EBRT of our experiment is only 50 s, which greatly reduces the residence time and has strong applicability.

3.2. Long-term Performance of BTF System

In the actual production process, the emission amount and emission concentration of the exhaust gas are adjusted according to the working conditions, and are not static. Therefore, in order to investigate the effect of different factors on the toluene removal effect and stability of the whole system during long-term operation, this experiment records and studies the stable operation process of the BTF system.

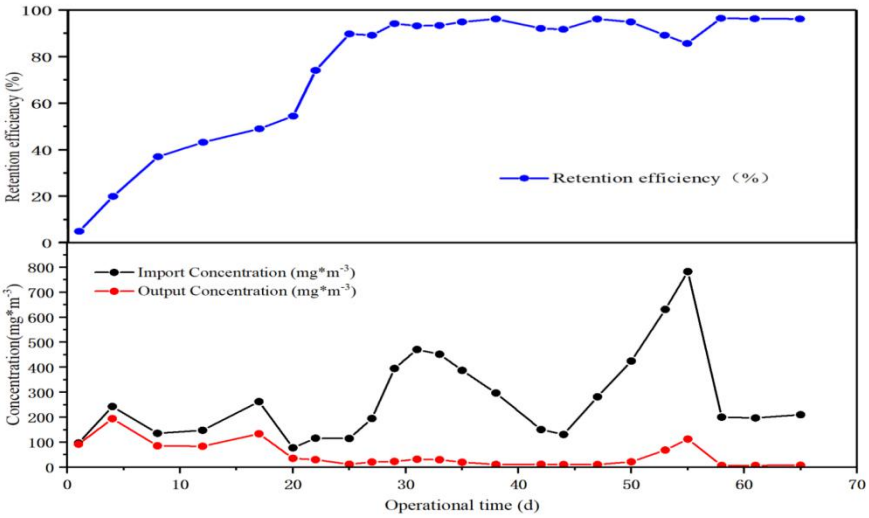


Figure 3: Performance of toluene removal efficiency during stable operation.

It can be seen from the data in Figure 3 that the system can operate stably after 30 days of biofilm culture. Even if the conditions such as toluene intake, toluene import concentration and nutrient spray rate are changed, the removal efficiency is basically stable within the range of 90%~97%. Only when the toluene import concentration is increased to 800 mg/m3, the removal efficiency is lower than 90% but higher than 85%. After reducing the import concentration, the toluene removal rate can quickly reach over 96%, which proves that the BTF system has a good stability.

3.3. BTF System Long-term Operation of Biofilm Morphology

Microbial community structure is a key indicator for evaluating the purification performance and stability of biological drip tower[4,8,13]. In order to understand the growth state and structure of the biofilm on the filler in this experiment, the microbial membrane attached to the filler was collected for scanning electron microscope observation on the 65th day of the stable operation of the bio-trickling system. The results of the correlational analysis are set out in Figure. 4. When observing photographs with diameters of 50 μm and 100 μm, it is obvious that the biofilm has a certain thickness and there is a gap between the layers; when observing a photograph with a diameter of 30 μm, the colony morphology can be visually observed, mainly Rod and spherical. From the scanning electron micrograph, some gaps can be observed, and the thickness of the biofilm can be known through these gaps, which indirectly indicates that the biofilm is in a mature state.

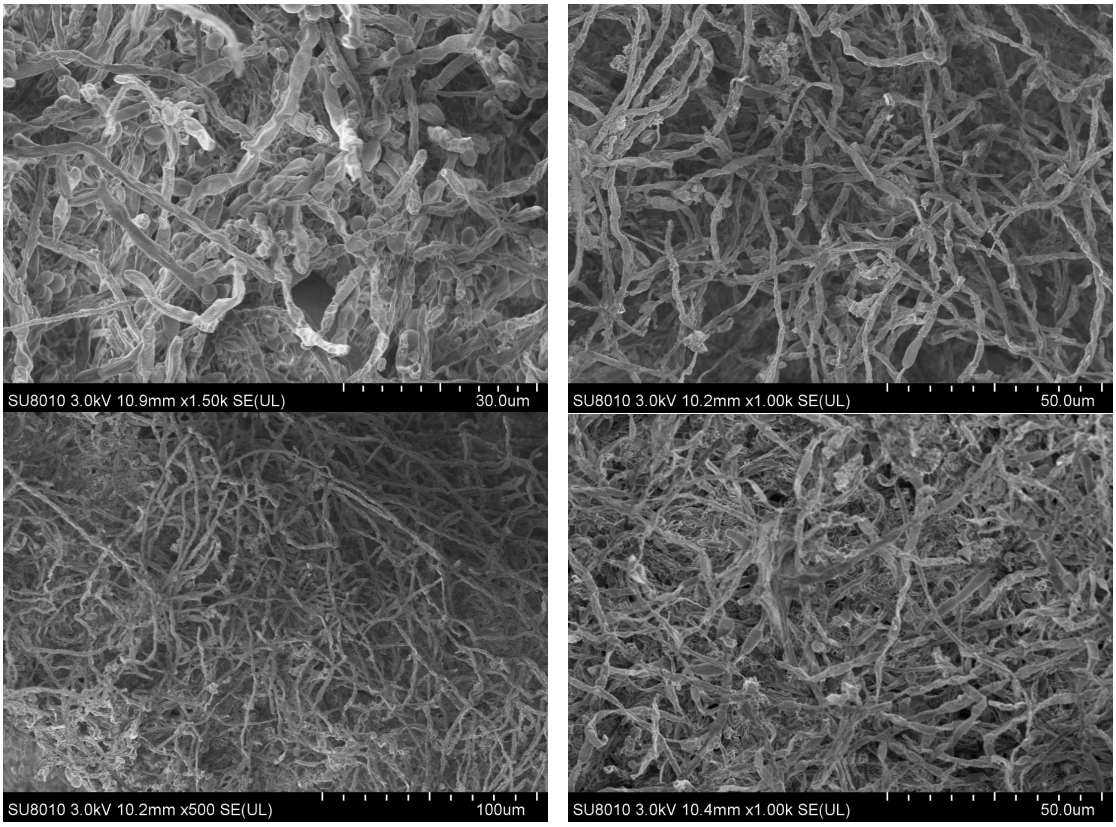


Figure 4: SEM of biofilm during stable operation.

3.4. Identification of Microbial Colonies in BTF System

3.4.1. Statistics on the Number of Microbial Groups at each Classification Level

Chao1 and ACE mainly show community richness, while Shannon and Simpson mainly reflect Community diversity. Calculate the above four diversity indices on the sample using QIIME software.

Table 3: Microbiota microbial diversity index.

Sample name	Simpson	Chao1	ACE	Shannon
1761	0.983319	1205.00	1205.00	7.72

Table 3 compares the intercorrelations among the four diversity indices of microbial colonies in stable operation, including Simpson index of 0.983319, Shannon index of 7.72, and Chao1 and ACE index of 1205.00. It indicates that the microbial community diversity is high and the community richness is at a high level.

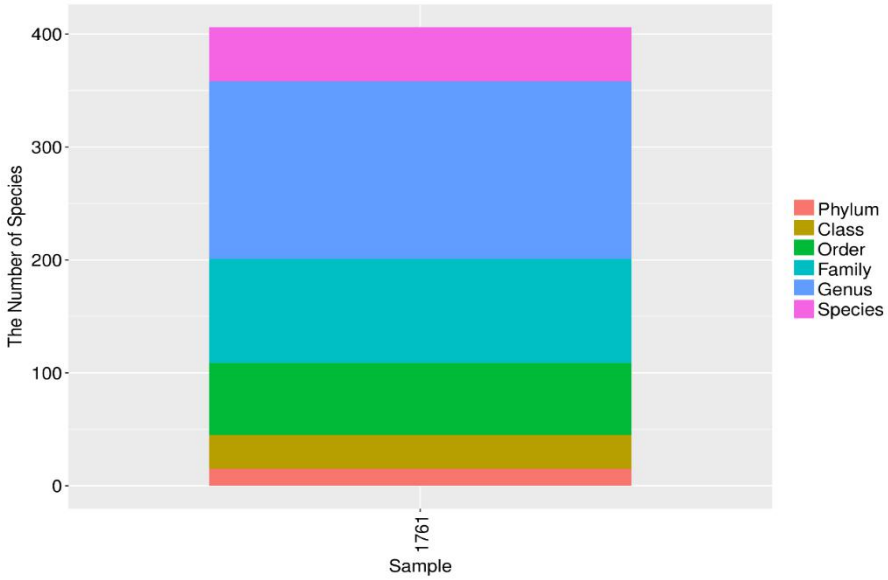
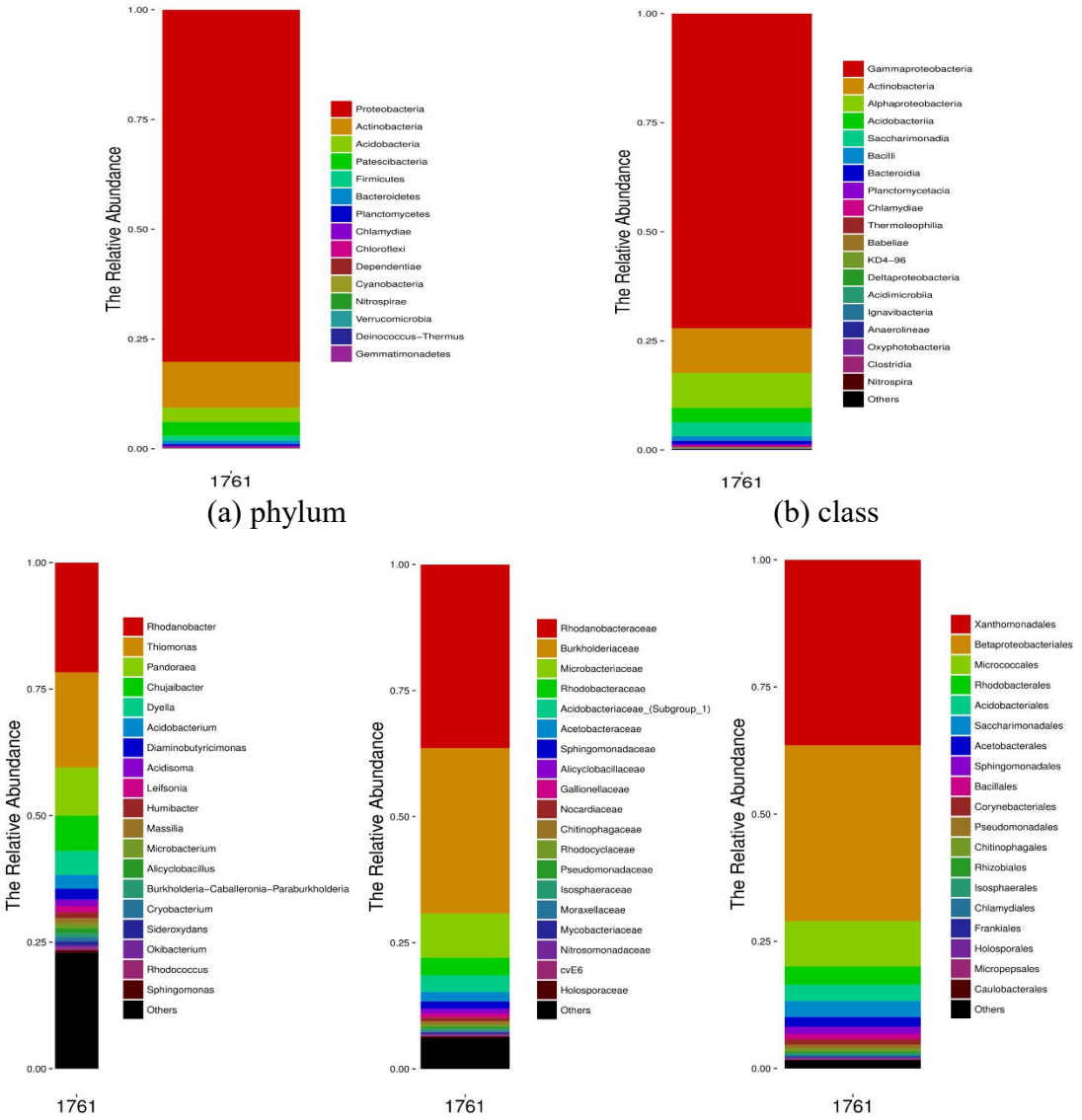


Figure 5: Number of microbial groups at each classification level.

By using R software to draw the statistical chart (Figure 5) of the number of microbial groups at each classification level, the difference in the number of taxonomic units at the same level and the number of microbial groups contained in each of the six taxonomic levels -- phylum, class, order, family, genus and species.

3.4.2. Analysis of Taxonomic Composition of each Classification Level

In order to analyze the composition and relative abundance distribution of the experimental sample at five levels of classification including phylum, class, order, family and genus, the QIIME software was used to draw a histogram of the colony of a single sample in the experiment(Figure 6).Proteobacteria occupies a dominant position in the Phylum category. In the Class category, Gammaproteobacteria accounts for a large proportion. Further analysis showed that Rhodanobacter and Thiomonas are the most abundant in genus category. Further statistical tests revealed Rhodanobacteraceae, Burkholderiaceae, Xanthomonadales, Betaproteobacteriales also play a key role in the processing of toluene by microorganisms.



(a) phylum (b) class

(c) genus (d). family (e). order

Figure 6: Taxonomic composition and distribution map of strains.

3.4.3. Phylogenetic Tree and Classification Level Tree Construction

The hierarchical tree drawn by GraPhlAn can not only distinguish the classification units from the Phylum to the Species in different colors, that is, from the inner circle to the outer circle, and can reflect the classification unit by the size of the node, that is, from the Phylum to the Species according to the outer layer to the inner The abundance distribution of the layers in sequence.

The colonies of the top twenty species with relative abundance include Proteobacteria, Actinobacteria, and Acidobacteria. Nearly 50% of the sequences belong to the proteobacteria, which is the most dominant population in the process of BTF operation. It is also found that Gammaproteobacteria is the largest of all the microflora(Figure. 7).

Using Krona software for interactive display of community taxonomic composition[15], compared to the above-mentioned GraPhlAn software, Krona can not only visualize the sample taxonomic composition, but also focus on the interactive display of data.

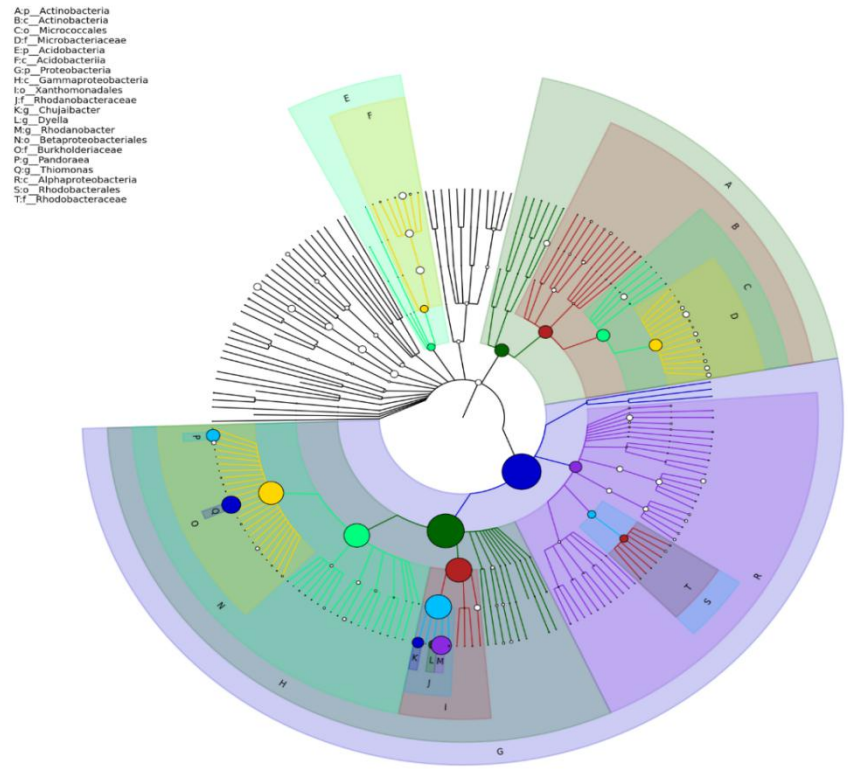


Figure 7: Sample overall classification level tree based on GraPhlAn

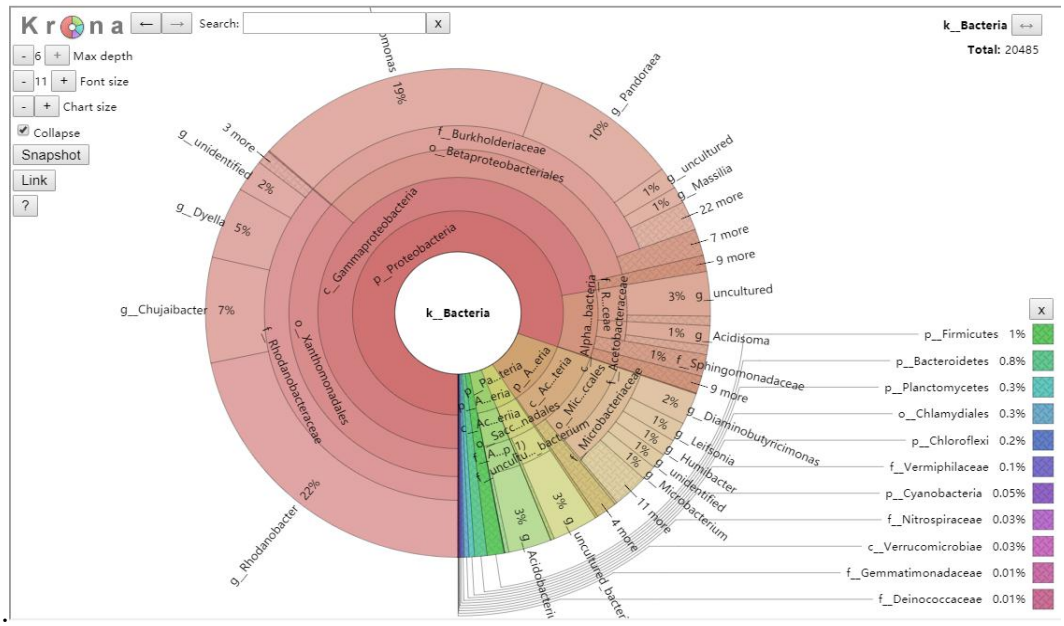


Figure 8: Krona based taxonomic composition information interactive display

As can be seen from Fig. 8, Dyella (5% abundance) belongs to Gammaproteobacteria, Burkholderiales (22%), rhizobium Rhodanobacter (22%), Thiomonas (19%), Pandoraea (10%). The strains all have a degradation or tolerance gene for toluene[17,21].

3.4.4. Functional Group Distribution Statistics

Figure 9 is a histogram of the abundance distribution of each functional group in the sample. It is based on the results of the PICRUSt prediction. The abscissa indicates the KEGG second-level functional group, and the ordinate indicates the relative abundance. As can be seen from the figure, the relatively abundant abundance is Amino Acid Metabolism, Carbohydrate Metabolism, Energy Metabolism, Metabolism of Cofactors and Vitamins. Lipid Metabolism, Nucleotide Metabolism, Xenobiotics Biodegradation and Metabolism, and the like.

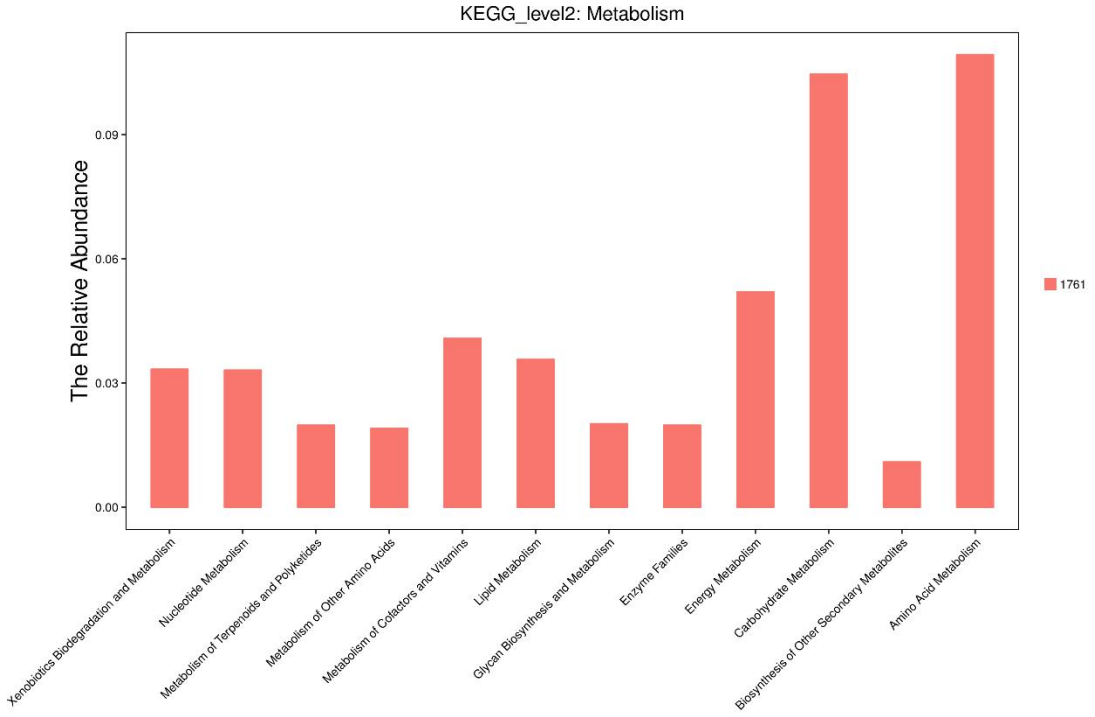


Figure 9: KEGG second level distribution predicted by PICRUSt.

4. Conclusions

(1) The control system temperature is 22-28 ° C, the toluene intake is 15 L/min, the toluene import concentration is 100~500 mg/m3 , the nutrient spray rate is 60 mL/min, and the toluene removal efficiency is stable at about 90% after 25 days. Under the condition that no exogenous strains are added, the biofilm culturing can be completed in 30 days, and the removal efficiency is maintained at about 90%.

(2) The BTF system can be operated stably after 30 days of biofilm culture. The removal efficiency is stable at about 90% by changing the toluene intake by 5~25 L/min, the toluene import concentration of 100~800 mg/m3 and the nutrient spray rate of 10~100 mL/min. The toluene import concentration increased to 800 mg/m3, and the removal efficiency decreased. However, after the concentration was lowered, the removal rate could quickly reach 96% or more, indicating that the BTF system has a small change under the condition of changing external environmental factors.

(3) Four kinds of diversity indices were used to identify microbial colonies during stable operation. The Simpson index was 0.983319, the Shannon index was 7.72, and the Chao1 and ACE indices were both 1205.00, indicating that the microbial community diversity was high and the community richness was also higher level.

(4) As the biofilm culturing is completed, the morphology of the biofilm changes from a sparse beginning to a dense microbial community. Bacterial colonies are attached to the hyphae and are mainly present in the form of bacillus and cocci. High-throughput analysis of microbial colonies showed that the microorganism in this experiment have high abundance and species diversity. Proteobacteria are the main dominant species, and Gammaproteobacteria is the most abundant of the proteobacteria. In addition, it was found that the Burkholderiaceae, Pandoraea, Rhodanobacter, Thiomonas, Dyella and other genus also played a key role in the degradation of toluene..

Acknowledgments

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